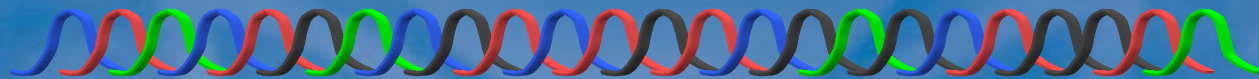


## ***2004 Science Forum***

# **Targeted screening for invasive species in ballast: genomic approaches**



**Michael Blum  
USEPA**

**Office of Research and Development  
National Exposure Research Laboratory**

**RESEARCH &  
DEVELOPMENT**

*Building a  
scientific  
foundation  
for sound  
environmental  
decisions*



**European green crab**

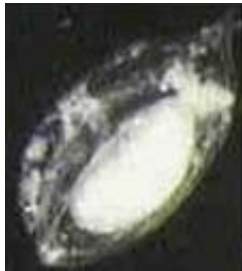


**Zebra mussels**



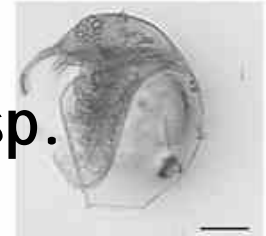
RESEARCH &  
DEVELOPMENT

*Building a  
scientific  
foundation  
for sound  
environmental  
decisions*



??

Daphnia sp.



Bosmina sp.



European green crab



Zebra mussel



Polychaete





# Identifying species found in ballast

## Morphological taxonomy

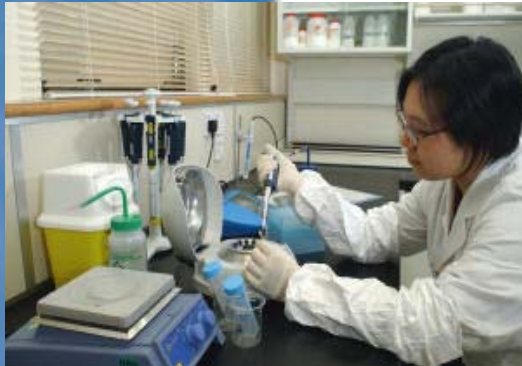
- traditional approach
- technologically simple (ie. microscopy...)
- classification dependent on adult traits
  - *larval and egg forms poorly characterized*
- requires broad knowledge of major taxonomic groups
  - *or requires assistance from a range of experts*
- *identification typically limited to family or genus level*
- *limited treatment of cryptic or difficult taxa*
- **no standard for comparison across studies**
- data have limited applicability (ie. species inventories...)



# Identifying species found in ballast

## Bioinformatics and “DNA taxonomy”

- alternative approach that may substitute for or complement traditional morphological taxonomy
- technology dependent on application (simple to complex)
- classification dependent on genomic variation
  - *useful for classifying eggs, larvae and adults*
- does not require broad knowledge of major taxonomic groups
  - *requires expert training in genetics and bioinformatics*
- *identification at the species or subspecies level*
- *full treatment of cryptic and difficult taxa*
- **provides an objective standard for comparison across studies**
- data have broad applicability



## ***Targeted screening of ballast for invasive species***

P.I. Michael J. Blum (blum.mike@epa.gov)  
EPA/ORD/NERL Cincinnati

---

- Research supported by the Regional Methods program
  - **ORD partnering with Regions 5, 9, 10 and GLNPO**
- Novel application of allele-specific PCR methods and DNA sequencing technology
- Development and application of bioinformatic databases
- Research objectives:
  - **Exploratory characterization of species diversity in ballast**
  - **Targeted screening of ballast for invasive species**

## *Potential applications*

- Early detection and monitoring of:
  - Non-indigenous species (NIS) of concern
  - Cryptic invasions
  - Introgressive hybridization between NIS and endemic species
- Assessing compliance with treatment requirements (ie. open-ocean exchange)
- Risk assessment
- Characterization of invasion events
  - Frequency
  - Magnitude
  - Directionality (pathways)
  - Post-introduction population dynamics

} “propagule pressure”

# Research objectives

- (1) Exploratory characterization of faunal species diversity found in ballast
- (2) Targeted screening for individual species of concern
  - Established species in the Great Lakes:
    - *Dreissena polymorpha*, *D. bugensis* (Zebra and Quagga mussels)
    - *Gymnocephalus cernuus* (Eurasian ruffe)
    - *Neogobius melanostomus* (Round goby)
    - *Proterorhinus marmoratus* (Tubenose goby)
    - *Cercopagis pengoi* (Fish-hook water flea)
    - *Bythotrephes longimanus* (Spiny water flea)
    - *Echinogammarus ischnus* (amphipod)
    - *Bosmina maritima* (cladoceran)
    - *Nitocra incerta* (copepod)
  - Species likely to invade the Great Lakes:
    - *Perca fluviatilis* (European perch)
    - Others...
  - Established species in Pacific estuaries and elsewhere:
    - *Carcinus maenas* (European green crab)
    - *Eriocheir sinensis* (Chinese mitten crab)



## ***Timeline and status of the project...***

- Initiated in December, 2003
- Ballast samples from Great Lakes secured through collaborators in February and May, 2004
- Contracts recently drafted to sample ship traffic between San Francisco, Columbia River and Puget Sound
- Laboratory work now underway on Great Lakes samples
- Sampling of Pacific coast traffic will commence in late summer, 2004 and will continue through Spring, 2005

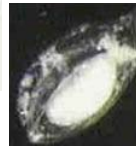
## RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions

Resting eggs or tissue in ballast water or sediment



[www.glerl.noa.gov/res/task\\_rpts/nsreid10-1.html](http://www.glerl.noa.gov/res/task_rpts/nsreid10-1.html)

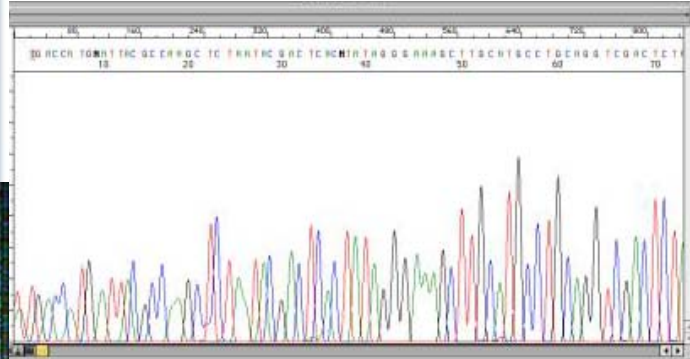
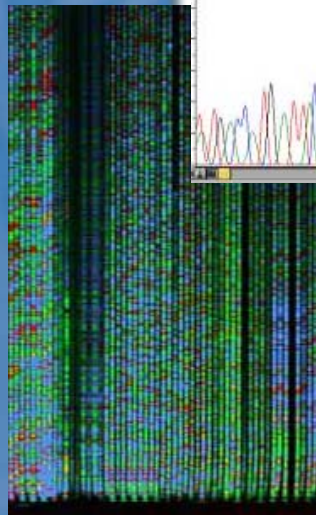


DNA extraction and purification



“from sludge to sequences”

Sequencing of cloned amplicons



Allele-specific PCR amplification



Bacterial cloning of amplicons



## Designing allele-specific PCR primers for preferential amplification of targeted species or groups of species

	Primer binding site conserved within target group	Amplicon consistent within target species	
Target species A individual 1	ACTGACAGCGGGCACTACG	ATTACATCGAGCATTATATGCGGCATT	
Target species A individual 2	.....	.....	
Target species A individual 3	.....	.....	
Target species B	.....	.....G.....C.....G.	Amplicon varies among target species
Target species C	.....	.....-.....T.....G.	
Excluded species X	.....-T..A.	.....T...A.....-...G.	
Excluded species Y	.....-..A.	.....T.....-...T.....	
Excluded species Z	.....-..TA.	.....T.....G.....-...G.	
	Primer binding site absent / ineffective in members of excluded group		

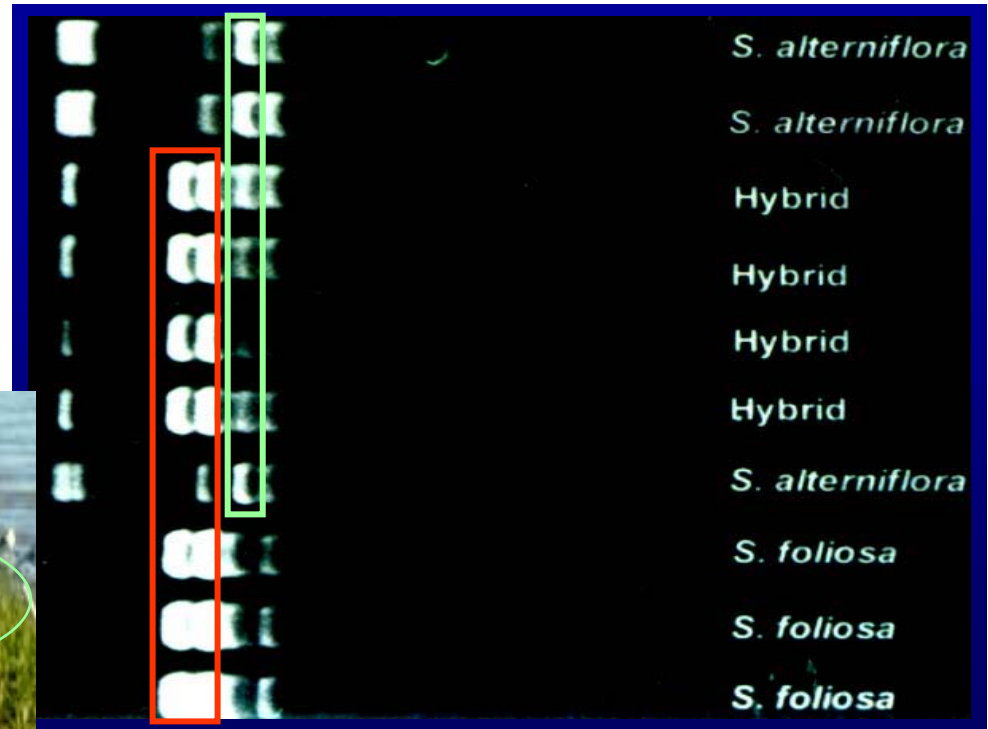
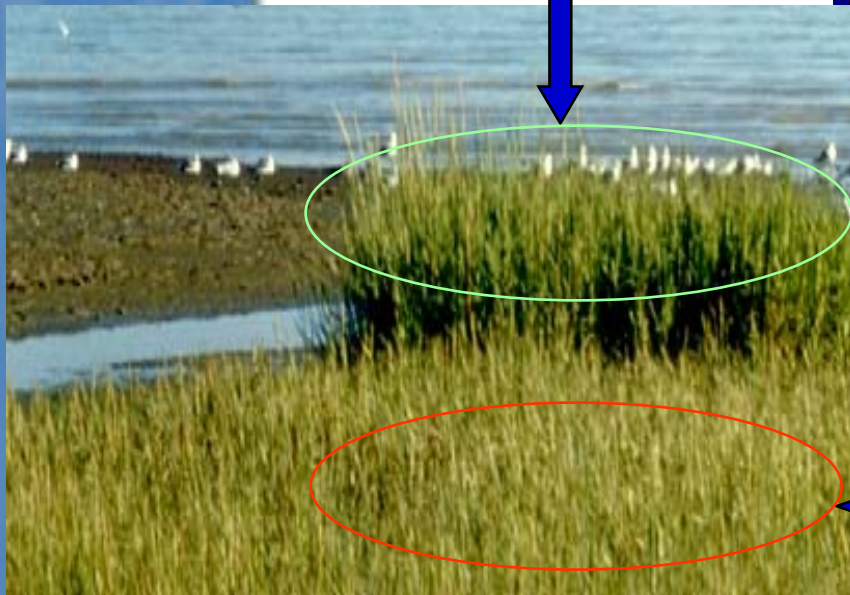
**Requires identification of primer binding sites that are:**  
**identical among individuals within a target group**  
**absent or ineffective among members of excluded group**

**Requires identification of amplicon gene regions that are:**  
**consistent within the target group**  
**variable among members of different target groups**



***Diagnostic markers can be used to differentiate  
between sister species, and even hybrids***

*Spartina alterniflora*



*Spartina foliosa*

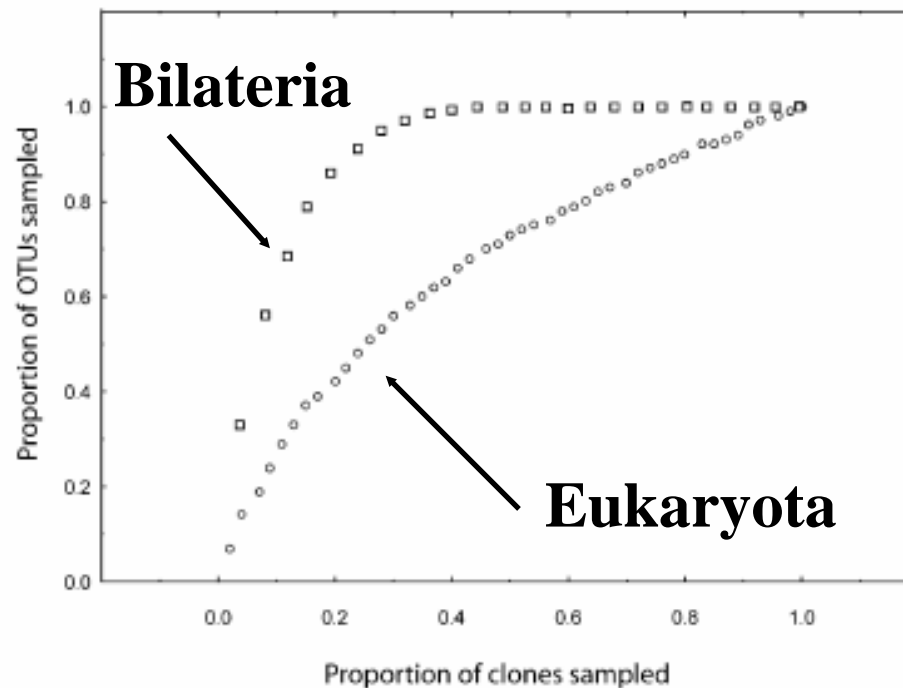






# DNA sequencing of cloned amplicons

To reduce costs, a subsample of cloned amplicons is sequenced



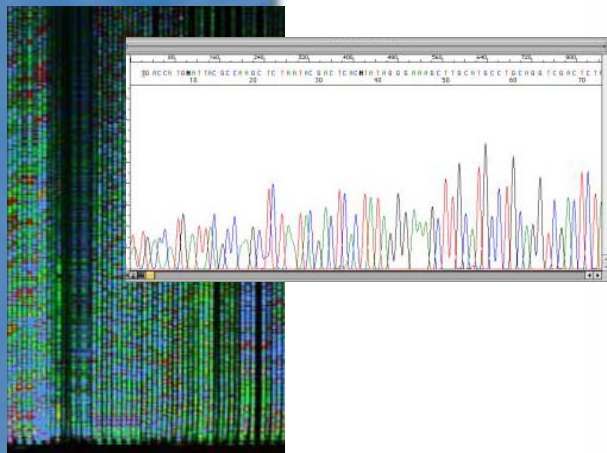
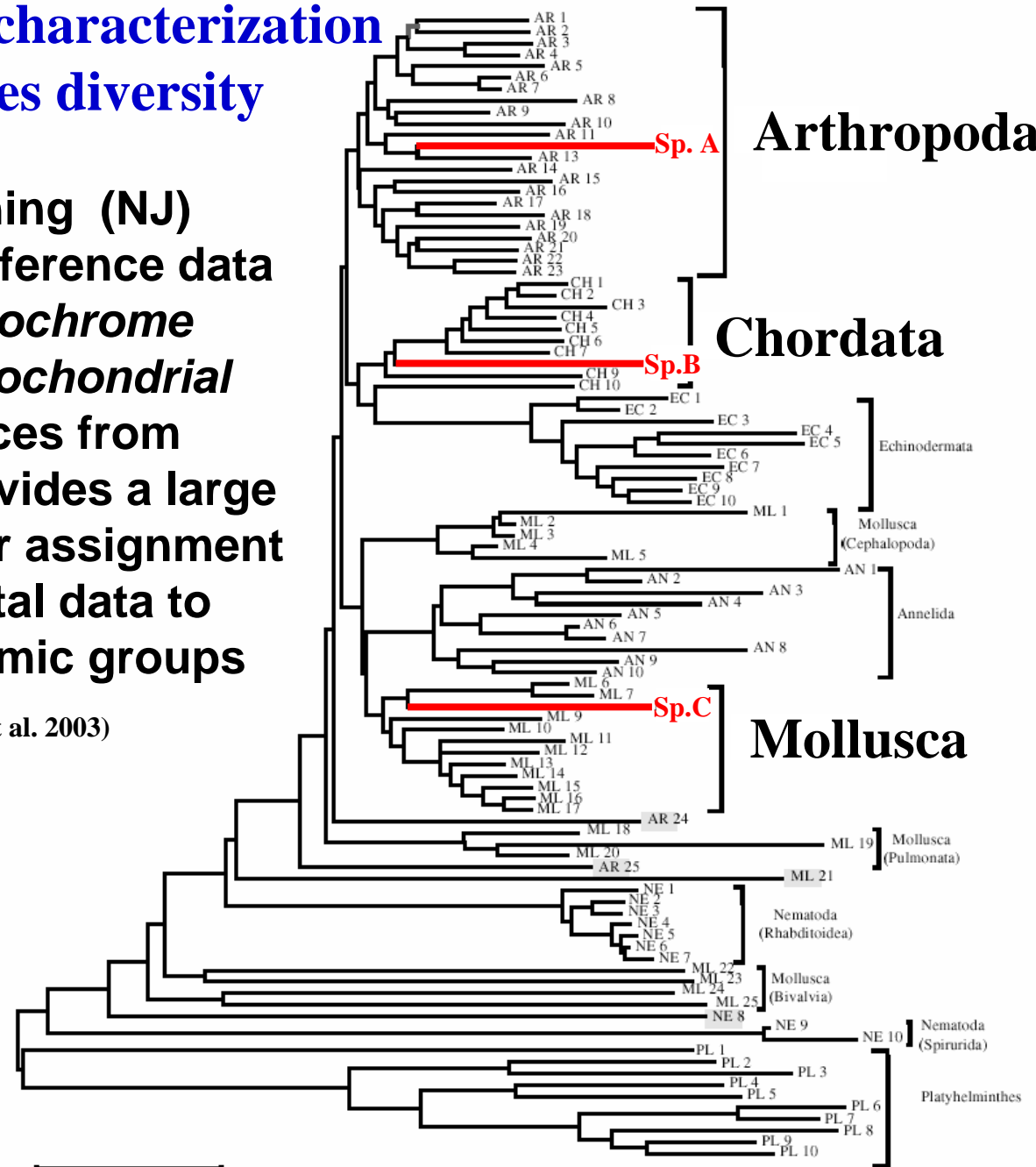
Jarman et al. 2004

The amount of sequencing necessary to sample all potential operational taxonomic units depends on the specificity of the target group- **less subsampling is required as specificity increases**

## Exploratory characterization of species diversity

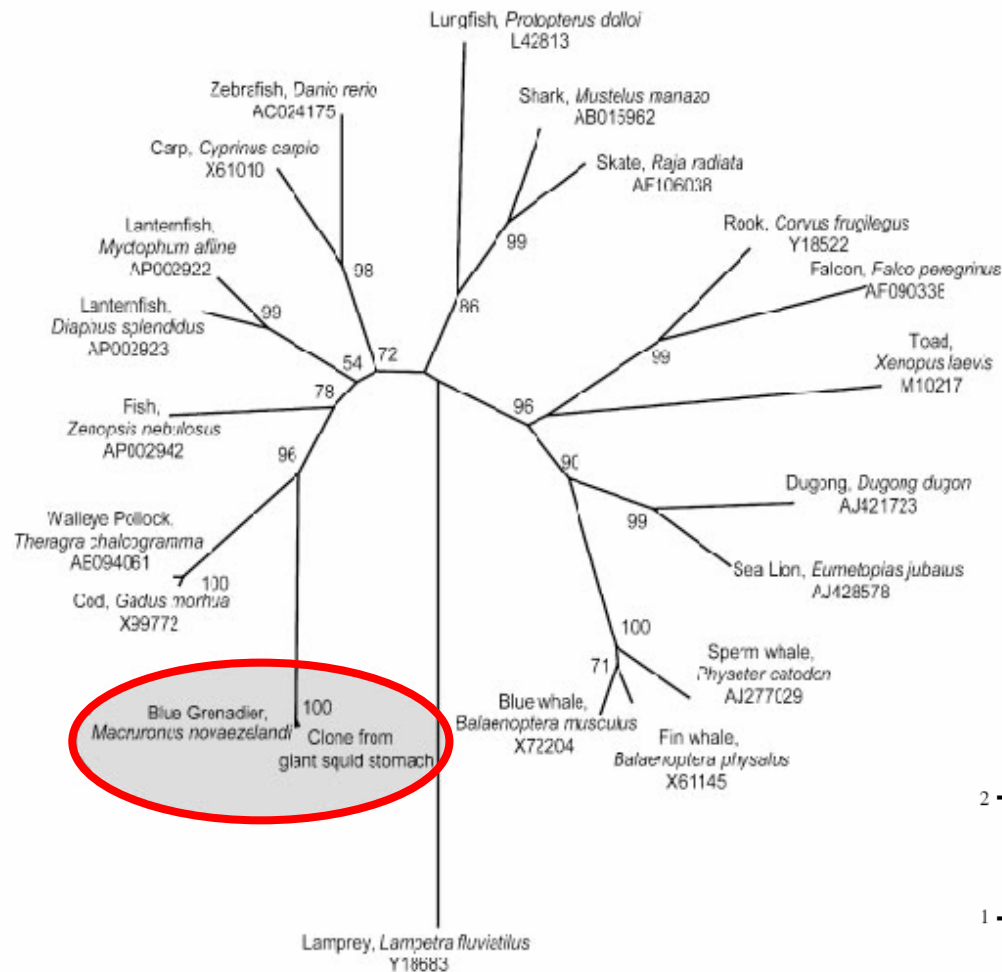
Neighbor-joining (NJ)  
analysis of reference data  
based on *Cytochrome  
Oxidase I mitochondrial  
gene* sequences from  
GenBank provides a large  
framework for assignment  
of experimental data to  
gross taxonomic groups

(redrawn from Hebert et al. 2003)



## RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions



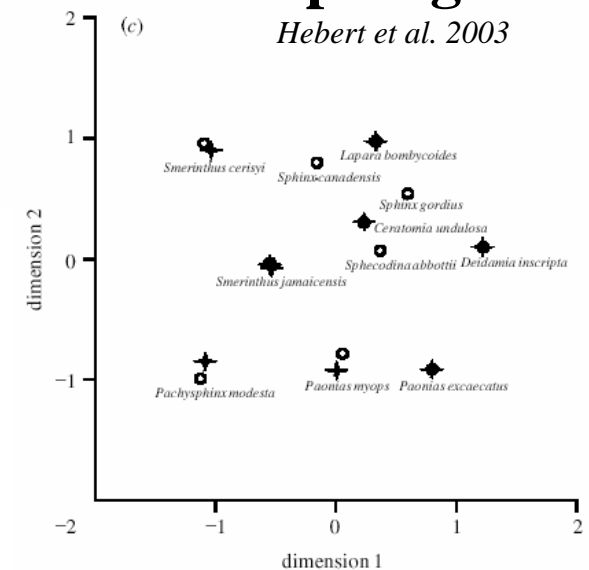
## Chordata

Jarman et al. 2004

Comparison of NJ and multidimensional scaling approaches for evaluating sequence specificity at fine taxonomic levels

## Sphingidae

Hebert et al. 2003



## ***Boiling it down...***

- **Molecular approaches can provide powerful tools for:**
  - **exploratory characterization of ballast water contents**
  - **targeted screening for species of concern**
    - consistently accurate species level identifications of all life stages
- **Molecular data function as a common denominator and have broad applicability**



## ***Future directions***

- **Application of techniques to support early detection and monitoring programs, assessing compliance with ballast treatment regs**
- **Development of stream-lined molecular tools for detection and monitoring (an invasive species gene array chip?)**
  - *May emerge through collaborations between EPA, USGS, USCG, NOAA and CSIRO*
- **Development and implementation of NIS-focused bioinformatic databases**
  - *An excellent opportunity to integrate morphological and DNA taxonomy approaches via collection of sequence data from curated specimens*
- **Further integration of multidisciplinary data (from genes to landscapes) to support risk assessment and vulnerability analyses of coastal regions**

RESEARCH &  
DEVELOPMENT

*Building a  
scientific  
foundation  
for sound  
environmental  
decisions*

# STOP BALLAST WATER INVASIONS



PERFORM  
OPEN OCEAN  
EXCHANGE IF  
SAFETY PERMITS

KEEP RECORDS  
OF BALLASTING  
OPERATIONS

REDUCE  
INVASIONS VIA  
HULL AND  
ANCHOR FOULING

MINIMIZE  
BALLASTING IN  
PORTS AND  
COASTAL AREAS

AVOID BALLAST  
UPTAKE  
AT NIGHT

AVOID BALLAST  
UPTAKE  
IN "HOT SPOTS"

## I N V A S I V E   S P E C I E S

<p>ASIAN CLAM</p>	<p>NORTHERN PACIFIC SEASTAR</p>	<p>CHOLERA BACTERIA</p>	<p>TOXIC DINO- FLAGELLATES</p>	<p>CHINESE MITTEN CRAB</p>	<p>NORTH AMERICAN COMB JELLY</p>
-----------------------	---	-----------------------------	------------------------------------	--------------------------------	--